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BRIEFER ARTICLES

MANIPULATING MICROSCOPIC ORGANISMS IN STAINING[1]

Perhaps the chief limiting factor in the successful preparation of permanent microscopic mounts of unicellular and colonial organisms is the difficulty of manipulation during the staining, with the incidental loss of the material in the necessary changing of the staining and washing solutions. The small size of such forms as *Sphaerella*, *Pandorina*, *Volvox*, *Pediastrum*, and the desmids renders these organisms especially liable to loss in these parts of the process. When handling the very small forms, the usual precautions taken in staining filamentous algae are generally wholly inadequate to prevent the loss of the material.

The difficulties in the way of success, however, may be overcome by using a funnel, filter paper, and a wash bottle, and combining the careful manipulations of quantitative chemistry, to prevent loss of valuable material, with the dehydration and staining methods of the Venetian turpentine method of mounting algae.[2] After the organisms have been killed in the usual 1:1:400 chromacetic acid solution, the entire contents of the vessel can be filtered, leaving the material to be stained on the filter paper in the funnel. Complete washing to remove the killing fluid can be secured by the use of the wash bottle. Should it be desirable to let the material stand in water for a few hours, the filter paper may be punctured and the material washed out into a beaker (this will save the work of washing). Refiltering later will leave the material on the filter paper and, with a little washing with water from the wash bottle, ready for the staining.

This manipulation is especially adapted to use with the iron-alum haematoxylin stain. The staining method itself may be greatly shortened, and in the modified form gives splendid results with these lower forms. The iron-alum should be dissolved in distilled water and all water treatments of material should be made with distilled water to avoid precipitates. Weak solutions of iron-alum should be used, 0.1 per cent solution at most. Frequently a few drops of a 1 per cent solution in 100 cc. of water are sufficient. The iron-alum solution is applied slowly to the material on the filter paper in the funnel. After a short time, 15–30 minutes or even less being sufficient, the material is washed thoroughly with distilled water from the wash bottle. A weak haematoxylin stain is then applied slowly to the material, and repeated observations of specimen mounts under the microscope will determine when the staining is complete. A 0.1 per cent haematoxylin stain is strong enough, and 30 minutes or less time is long enough for it to act.

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[1] Contribution from the Department of Botany, Pennsylvania State College, no. 8.

[2] CHAMBERLAIN, CHAS. J., Methods in plant histology, 3d ed. Chicago, 1915.

When the staining is complete, the differentiation of the stain is accomplished by a thorough washing in distilled water and the customary destaining with the 0.1 per cent iron-alum solutions. This latter step is the one requiring the greatest care, since it can be overdone most easily; the light haematoxylin stain used in the method is easily lost by prolonged treatment with even a very weak iron-alum solution. One application of the destaining solution is generally sufficient if care be taken that the material is thoroughly saturated with the solution. As usual when using the iron-alum solution for differentiating a stain, the process should be closely observed under the microscope. It is to be understood that no directions concerning the time limits can be given for the use of either the staining or differentiating solutions, since success depends upon the proper balancing of these two processes. When the stain has been properly differentiated, the material should be thoroughly washed with distilled water from the wash bottle. At any stage of the process, should it be desired to allow the material to remain covered by the solution being used, this can be accomplished by fitting a short piece of rubber tubing over the stem of the funnel and using a clamp to stop the flow of the solution.

Dehydration is accomplished by the glycerine dehydration method in general use. The transfer to the 5 per cent glycerine solution is made by puncturing the filter paper and washing with the glycerine solution into an open vessel, such as a Petri dish. The minimum amount of glycerine should be used, since it is difficult to remove. After a few days' exposure to evaporation, the glycerine solution is concentrated. The material is again poured into the filter paper in the funnel and complete dehydration accomplished by washing all the glycerine out with 95 per cent alcohol, followed by absolute alcohol to complete dehydration. There should be no doubt about the thoroughness of this part of the process, since complete dehydration is essential to success. When completely dehydrated the material is transferred quickly to a 10 per cent Venetian turpentine solution and placed in a desiccator and allowed to concentrate according to the directions given for the Venetian turpentine method.

Should it be desired to use some of the other stains, such as the Magdala red-anilin blue combination recommended for algae, it will be necessary to modify this manipulation to suit the method. Since these stains are used in strong alcoholic solutions, the material to be stained is washed after killing by the method already described, and then dehydrated by the glycerine method before staining. The glycerine is washed out with 95 per cent alcohol and the stains applied.

Summary

1. Treat the material the proper length of time in a suitable killing solution.
2. Filter the material to remove killing solution, leaving the material on the filter paper in the funnel.
3. Wash with distilled water from a wash bottle.
4. Treat with a 0.1 per cent (or less) iron-alum solution.
5. Wash with distilled water, using wash bottle.
6. Stain by application of 0.1 per cent (or less) aqueous haematoxylin stain.
7. Wash with distilled water.
8. Differentiate the stain with 0.1 per cent iron-alum solution, washing with distilled water very thoroughly after the treatment.
9. Dehydrate with glycerine and mount by Venetian turpentine method.
10. Vary the treatment, when alcoholic stains are to be used, by dehydrating before staining.—J. BEN HILL, *Pennsylvania State College, State College, Pa.*

THE BOTANICAL STATION AT CINCHONA

The Botanical Station at Cinchona, in the Blue Mountains of Jamaica, which from 1903 to 1913 was leased by the New York Botanical Garden, has now been leased by the Smithsonian Institution, on behalf of 14 American botanists and botanical institutions that have contributed the rental. These botanists and institutions believe that there is need in the American tropics for a counterpart of the famous Buitenzorg Garden of Java. They hope that the opening of this laboratory at Cinchona may prove as stimulating to the development of botany in